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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/819,266	03/28/2001	Agamemnon Antoniou Epenetos	JG-EPC-4955P/500563.20004	4300

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EXAMINER
DAVIS, MINH TAM B

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 08/27/2003

17

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/819,266	EPENETOS, AGAMEMNON ANTONIOU	
	Examiner	Art Unit	
	MINH-TAM DAVIS	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-27* is/are pending in the application.

4a) Of the above claim(s) *2,4-9,17-20 and 23-27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,10-16,21 and 22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Accordingly, claims 1, 3, 10-16, 21 and 22 are examined in the instant application.

OBJECTION

1. Claim 1 is objected for the use of the language "portionis". Does Applicant mean "portion is"?

2. Claim 14 is objected to for the use of the language " a poptosis". It is not clear what " a poptosis" is. Does Applicant mean "apoptosis"?

3. Claims 21-22 are objected to, because claims 21-22 depend on non-elected claims 2, 4-9.

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION, NEW REJECTION

The amended claims 1, 3, 10-16, 21-22 are rejected under 35 USC 112, first paragraph, pertaining to lack of a clear written description of "a portion for binding to a specific target cell", for reasons already of record in paper No:14.

Claims 1, 3, 10-16, 21-22 are drawn to a compound comprising "a portion for binding to a specific target cell" and a cytotoxic portion, which is a constitutively active

caspase or has the same apoptosis-inducing activity as said caspase, wherein the cytotoxic portion is conjugated to a portion specific for binding to a cell.

The specification only discloses, as an example of target cell specific portion, antibodies or fragments thereof that bind to various antigens or receptors (p.14-23).and a conjugate of an antibody fragment scFv against CEA and a rearranged, constitutively active caspase (p.57-58 and figure 9).

It is noted that "a portion for binding to a specific target cell" however comprises any compound with any structure that binds to a target cell, such as various ligands with various structure for receptors on the surface of target cells, the structure of which is completely different from that of an antibody or an antibody fragment scFv and is not disclosed in the specification.

Although drawn specifically to the DNA art, the findings of *The Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412) are clearly relevant to the instant rejection. The court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that while Applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. At section B(1), the court states that "An adequate written description of a DNA...'requires a precise definition, such as by structure, formula, chemical name, or physical properties', not a mere wish or plan for obtaining the claimed chemical invention".

No common structural attributes that identify the claimed "portion for binding to a specific target cell" are disclosed. In addition, no common functional attributes that identify the claimed "portion for binding to a specific target cell" are disclosed, because the function of a polypeptide sequence could be abolished, even with substitution of only one amino acid of the polypeptide (Burgess et al, of record).

Since the disclosure fails to describe the common attributes or characteristics that identify members of the claimed "portion for binding to a specific target cell", an antibody and its antigen binding fragments thereof alone is insufficient to describe the claimed variants. One of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of variants. Thus, applicant was not in possession of the claimed nucleotide sequences encoding said variants.

Thus, there is insufficient support of claims 1, 3, 10-16, 21-22 as provided by the Interim Written Description Guidelines published in the June 5, 1998 Federal Register at Volume 63, Number 114, pages 32639-32645. Therefore, only a compound comprising and an antibody specific for a target cell and a cytotoxic portion, which is a constitutively active caspase or has the same apoptosis-inducing activity as said caspase, wherein the cytotoxic portion is conjugated to said antibody, but not the full breadth of the claims meets the written description provision of 35 USC 112, first paragraph.

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH, SCOPE

1. Claims 1, 3, 10-16, 21-22 remain rejected under 35 USC 112, first paragraph, pertaining to lack of enablement for a compound comprising "a portion for binding to a

specific target cell" and a cytotoxic portion, for reasons already of record in paper No: 14.

Applicant amends the claims to recite a compound comprising "a portion for binding to a specific target cell" and a cytotoxic portion.

Rejection remains, because the claims encompass any compound that binds to a specific target cells, wherein one cannot predict whether said compound would be internalized and whether the claimed conjugate would be delivered to proper cellular compartment where caspase exerts its action.

Moreover, the specification only discloses, as an example of a target cell specific portion, antibodies or fragments thereof that bind to various antigens or receptors (p.14-23) and a conjugate of an antibody fragment scFv against CEA and a rearranged, constitutively active caspase (p.57-58 and figure 9). MPEP 2164.08(a) however teaches that a single means claim which covered every conceivable means for achieving the stated purpose was held nonenabling for the scope of the claims because the specification disclosed at most only those means known to the inventor. *In re Hyatt*, 708 F.2d 712, 714-715, 218 USPQ 195, 197 (Fed. Cir. 1983). In the instant application, the specification only discloses a single example of a target cell specific portion, antibodies or fragments thereof that bind to various antigens or receptors. . However, the scope of the claim encompasses a compound comprising a portion for binding to a specific target cell and a cytotoxic portion, wherein said portion has any structure and function, provided it binds to specific target cells. Thus the claims would be non-enabled according to MPEP 2164.08(a).

2. Claims 1, 3, 10-16, 21-22 remain rejected under 35 USC 112, first paragraph, pertaining to lack of enablement for a compound comprising a portion for binding to a specific target cell and a cytotoxic portion, which is "any constitutively active caspase, or has the same apoptosis-inducing activity as said caspase", for reasons already of record in paper No: 14.

Applicant argues that the definition of the constitutively active caspase is found at page 3, line 18 to page 7, line 4, as being "a caspase in an activated form" or "a precursor of an active caspase that can spontaneously self-catalyse to the active caspase". Applicant further asserts that the test for apoptotic activity is described in Example H of the specification.

It is noted that a cytotoxic portion "having the same apoptosis-inducing activity as a constitutively active caspase" encompasses any compound provided it induces apoptosis. In other words, the claimed compound encompasses for example a conjugate of any wild type effector caspases, including procaspase-3, which could produce apoptosis, when activated. Colussi et al (of record) however teach that effector caspases such as procaspase-3 are poor inducers of cell death, when transfected into mammalian cells, presumably because of its inability to autoactivate. Thus it is unpredictable that a compound comprising a portion for binding to a specific target cell and a cytotoxic portion, which has the same apoptosis-inducing activity as a constitutively active caspase, i.e. any caspase, would be useful for anything, in view of their being ineffective in inducing apoptosis. In view of the above, it would be undue experimentation for one of skill in the art to use the claimed invention.

It is further noted the constitutively active caspase is defined in the specification as being "a caspase in an activated form". In other words, the claims encompass a conjugate of an activated caspase with a portion for binding to a specific target cell. It is questionable that a conjugate of an activated caspase is of any practical use. It is well known in the art that after activation, the activated caspase would expose its active serine protease site, which could be bound by a protease inhibitor. For example, Grabarek J et al, Experimental hematology (Netherlands) Sep 2002, 30 (9) p982-9 teach that only cells undergoing apoptosis are labeled with inhibitors of caspases and serine proteases, wherein said inhibitors bind to the enzymatic center or active sites of caspases and serine proteases. In other words, only activated caspases, but not procaspases, would expose their active serine protease site, and bind to the inhibitors of caspases or serine proteases. It is also well known in the art that there exists a family of serine protease inhibitors in cells and in blood, which could readily form a stable complex with serine proteases (Stief Thomas, 2000, Thrombosis Res, 98(6): 541-547, Djie Marylyn Z et al, J biological Chemistry 272 (26):p16268-16273 199, Bjartell A, 1993, Urology, 42 (5): 502-10), and Schimmer, AD, 2003, Cancer Res, 63(6): 1242-8). Thus one would expect that the claimed conjugate of an activated caspase with a portion for binding to a specific target cell would be readily bound to a caspase inhibitor or serine protease inhibitor and form a stable complex, before even reaching the proper site for its action.

Moreover, MPEP 2164.08(a) teaches that a single means claim which covered every conceivable means for achieving the stated purpose was held nonenabling for the

scope of the claims because the specification disclosed at most only those means known to the inventor. *In re Hyatt*, 708 F.2d 712, 714-715, 218 USPQ 195, 197 (Fed. Cir. 1983). In the instant application, the specification only discloses a single example of a "constitutively active caspase", i.e. caspase-3 and –6 variants taught by Srinivasula et al, 1998, wherein the subunits positions are reversed and rearranged, and wherein, different from the wild types, said variants are capable of autocatalytic processing. However, the scope of the claim encompasses a compound comprising a target cell-specific portion and a cytotoxic portion, wherein the cytotoxic portion is any active caspase or any apoptosis inducing compound. Thus the claims would be non-enabled according to MPEP 2164.08(a).

In view of the above, it would be undue experimentation for one of skill in the art to use the claimed invention.

3. Claims 1, 3, 10-16, 21-22 remain rejected under 35 USC 112, first paragraph, pertaining to lack of enablement for a compound comprising a portion for binding to a specific target cell and a cytotoxic portion, which is a constitutively active caspase " or has the same apoptosis-inducing activity as said caspase", i.e. any cell death agonist, for reasons already of record in paper No: 14.

Applicant argues that the definition of the constitutively active caspase is found at page 3, line 18 to page 7, line 4, as being "a caspase in an activated form" or "a precursor of an active caspase that can spontaneously self-catyalyse to the active caspase". Applicant further asserts that the test for apoptotic activity is described in Example H of the specification.

It is noted that a cytotoxic portion having the same apoptosis-inducing activity as a constitutively active caspase encompasses any compound provided it induces apoptosis. In other words, the claimed compound encompasses any cell death agonist, such as BAR, BAD and BAX.

It is unpredictable that a compound comprising a portion for binding to a specific target cell and a cytotoxic portion, which is any cell death agonist would be able to induce apoptosis, due to homeostasis or complex interaction between different members of the cell death agonists and antagonists, as taught by Gottschalk et al, of record.

Moreover, MPEP 2164.08(a) teaches that a single means claim which covered every conceivable means for achieving the stated purpose was held nonenabling for the scope of the claims because the specification disclosed at most only those means known to the inventor. *In re Hyatt*, 708 F.2d 712, 714-715, 218 USPQ 195, 197 (Fed. Cir. 1983). In the instant application, the specification only discloses a single example of a "constitutively active caspase", i.e. caspase-3 and –6 variants taught by Srinivasula et al, 1998, *supra*, wherein the subunits positions are reversed and rearranged, and wherein, different from the wild types, said variants are capable of autocatalytic processing. However, the scope of the claim encompasses a compound comprising a target cell-specific portion and a cytotoxic portion, wherein the cytotoxic portion is any apoptosis inducing compound. Thus the claims would be non-enabled according to MPEP 2164.08(a).

4. Claims 21-22 remain rejected under 35 USC 112, first paragraph, pertaining to lack of enablement for a pharmaceutical composition or a compound for use in medicine, for reasons already of record in paper No: 14.

Applicant argues that the Johnstone et al reference cited as rending claim 21 obvious clearly teach that it is common practice to formulate a pharmaceutical compositions.

Applicant argues that there is no evidence that shows in vitro results will not be repeated in an in vivo situation in the context of the present invention. Applicant argues that the in vitro data in the present invention is highly successful, i.e. 70% killing, even when a poorly internalized antibody (anti-CEA) is used. Applicant asserts that more readily internalized antibodies should be even more effective in vitro, and there is no reasons that the internalization of the antibody would be impaired in vivo. Applicant asserts that in the '894 patent recited by the Examiner, the disclosed immunotoxin in internalized both in vitro and in vivo, and cell death is effective in both experimental models.

Applicant's arguments in paper No:16 have been considered but are found not to be persuasive for the following reasons:

It is noted that due to the language "a pharmaceutical composition" or "a compound for use in medicine" the claims encompass a compound for use in vivo for "treating cancer".

Further, it is noted that the reference by the Johnstone et al is cited only to render obvious "the pharmaceutically acceptable carrier or excipient" which

encompasses buffer, water etc..., and not a pharmaceutical composition, which is recited as an intention for use, and is not given patentable weight in comparing the claims with the prior art.

Thus the issues encompass not only internalization of the claimed compounds, but also whether the claimed compound is stable in vivo, and even if the claimed compound reaches the target cells in an adequate amount, whether apoptosis would occur in vivo due to possible homeostasis, and to the unpredictability of cell responses in vivo and whether cancer is treated due to the unpredictability of cancer treament.

An immunoconjugate must accomplish several tasks to be effective. It must be delivered into the circulation that supplies the tumor and interact at the proper site of action and must do so at a sufficient concentration and for a sufficient period of time. In addition the target cell must not have a alternate means of survival despite action at the proper site for the drug. *In vitro* assays cannot duplicate the complex conditions of *in vivo* therapy. In the assays, the anti-tumor agent is in contact with cells during the entire exposure period. This is not the case *in vivo*, where exposure tat the target site may be delayed or inadequate. In addition variables such as biological stability, half-life or clearance from the blood are important parameters in achieveing successful therapy. The immunoconjugate may be inactivated *in vivo* before producing a sufficient effect, for example, by proteolytic degradation, immunological activation or due to an inherently short half life of the protein and the *in vitro* tests of record do not sufficiently duplicate the conditions which occur *in vivo*. In addition, the immunoconjugate may not otherwise reach the targert because of its inability to penetrate tissues or cells where its activity is

to be exerted, may be absorbed by fluids, cells and tissues where the immunoconjugate has no effect, circulation into the target area may be insufficient to carry the immunoconjugate and a large enough local concentration may not be established.

Further, it is unpredictable that apoptosis would occur in vivo, because cells in vivo have different characteristics and responses than cells in vitro, and because of possible homeostasis, and cell-cell interaction which do not exist in in vitro conditions, as taught by Drexler et al, Embleton et al, Hsu et al, Freshney et al, Dermer et al, all of record.

Moreover, it is unpredictable that the claimed compound could be used for treating cancer, because it is well known in the art that cancer treatment is unpredictable, as taught by Gura et all, Jain et al, Curti et al, and Hartwell et al, all of record. Further, Schimmer, AD, 2003, Cancer Res, 63(6): 1242-8 teach that cancer cells such as leukemia could overepxress endogenous inhibitors of the effector caspases, and block the caspase pathways. Thus it is unpredictable that the claimed compound could be useful for treating cancer, due to possible inhibition by the overexpression of inhibitors the effector caspases in cancer patients.

5. Claim 14 remains rejected under 35 USC 112, first paragraph, pertaining to lack of enablement for a "constitutively active variant" of a naturally occurring caspase, having apoptosis inducing activity, for reasons already of record in paper No: 14.

Rejection remains, because Applicant has not answered to this issue.

The claims encompass variants of a naturally occurring caspase, having deletion, substitution, and addition at any position of the naturally occurring caspase. Applicant

has not taught how to make said variants which are capable of functioning as that which is being disclosed, especially in view of the unpredictability of protein chemistry, wherein a single amino acid substitution of what appear to be an inconsequential chemical modification will often dramatically affect the biological activity and characteristic of a protein, as taught by Burgess et al, Lazar et al, Tao et al and Gillies et al.

ANSWERS TO APPLICANT'S ARGUMENTS AGAINST 103 REJECTION

Applicant argues that it is not obvious to replace ricin A of the immunotoxin of '894 patent with caspases as described in Srinivasula et al because ricin A and caspases are not equivalent compounds. Applicant asserts that ricin A acts by a different mechanism than caspases. Applicant asserts that ricin A acts by preventing protein synthesis, whereas caspases kill cells by actively cleaving and disrupting chromosomal DNA, the cytoskeleton and multiple enzymes.

Applicant argues that Colussi et al teach away from the invention, because Colussi et al teach that oligomerisation is essential for activation of caspases including caspase 3. Applicant asserts that the constitutively active caspase of the claimed invention require no oligomerization in order to be activated.

Applicant argues that Srinivasula only teach that the constitutively active caspases may be considered for use in target gene therapy, and that the gene base therapy is at odds with the protein based approach of the ricin A containing immunotoxin of '894 patent.

Applicant recites Marcelli et al, Hay et al, Vellera et al, Shariat et al, Shinoura et al, and Komata et al, all teach gene therapy using caspases. Applicant asserts that since the priority date to the present application, the technical population continues to have a prejudice toward gene delivery based methods. Applicant argues that one would not consider the disclosure of a possible gene therapy approach with a protein delivery approach, since there was not a reasonable expectation that such an approach would work.

The recitation of Marcelli et al, Hay et al, Vellera et al, Shariat et al, Shinoura et al, and Komata et al is acknowledged.

Applicant's arguments in paper No:16 have been considered but are found not to be persuasive for the following reasons:

It is noted that contrary to Applicant's arguments, ricin A from a conjugate of ricin A-antibody could induce apoptosis of target cells via activating a caspase, besides killing cells via arresting protein synthesis caused by inactivation of the elongation factor 2, as taught by Keppler-Hafkemeyer A, 1998, Biochemistry, 37(48): 16934-42.

It would have been obvious to replace ricin A, an apoptosis inducing compound, with another apoptosis inducing compound, i.e. the constitutively active caspase taught by Srinivasula et al, because using antibody for delivering a cytotoxic compound for killing target cells is well known in the art.

Moreover, Colussi et al do not teach away from the invention, because although Colussi et al teach that oligomerisation is essential for activation of caspases including caspase 3, the fusion protein taught by Colussi et al is an autoactivating caspase, and

thus is the same as the claimed constitutively active caspase, which is defined in the specification of the claimed invention as "a precursor of an active caspase that can spontaneously self-catalyse to the active caspase". In addition, concerning Applicant's assertion that the constitutively active caspases of the claimed invention require no oligomerization in order to be activated, Applicant argues limitation not in the claims.

Further, using antibody as a carrier for targeting a compound to specific cells is well known in the art. Thus it would have been obvious to make an antibody conjugate of the constitutively active caspase taught Srinivasula et al for the purpose of targeting to target tissues, as taught by Srinivasula et al, since the constitutively active caspase taught Srinivasula et al could be used at a very low concentration to induce apoptosis in target cells.

Further, although since the priority date of the present application, the technical population teaches gene delivery based methods for caspases, this does not make the claimed invention non-obvious, because protein based methods are complementary to the gene therapy methods.

One of ordinary skill in the art would have been motivated to make an antibody conjugate of the constitutively active caspase taught Srinivasula et al with a reasonable expectation of success in making such a conjugate, and in using said conjugate for killing target cells in vitro, since the constitutively active caspase taught Srinivasula et al could be used at a very low concentration to induce apoptosis in target cells, as taught by Srinivasula et al and since a conjugate of antibody-ricin A could also kill target cells by apoptosis, as taught by Keppler-Hafkemeyer A et al.

REJECTION UNDER 35 USC 103, NEW REJECTION

Claims 1, 3, 10-16, 21-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Srinivasula et al, 1998, of record, in view of US 4,753,894, of record, Colussi, PA et al, 1998, of record, and Keppler-Hafkemeyer A et al, 1998, Biochemistry, 37(48): 16934-42..

Claims 1, 3, 10-16 are drawn to for a compound comprising a fusion protein of a target cell-specific portion and a cytotoxic portion, wherein the cytotoxic portion is a constitutively active caspase or a constitutively active variant of a naturally occurring caspase, or has substantially the same apoptosis-inducing activity as the caspase. The target cell-specific portion recognizes and selectively binds to a tumor cell antigen. The cytotoxic portion is a constitutively active effector caspase-3. The cytotoxic portion is of mammalian origin and is capable of oligomerization.

Claims 21 and 22 are drawn to a pharmaceutical composition or a compound for use in medicine, comprising a fusion of a target cell-specific portion and a cytotoxic portion, wherein the cytotoxic portion is a constitutively active caspase, or has substantially the same apoptosis-inducing activity as the caspase.

Claims 21-22 recite the claimed compound formulated in a pharmaceutical composition or a compound for use in medicine. However, this limitation is viewed as a recitation of intended use and therefore is not given patentable weight in comparing the claims with the prior art. The claims read on the ingredient *per se*, which is a fusion protein comprising a target cell-specific portion and a cytotoxic portion, wherein the

cytotoxic portion is a constitutively active caspase, or has substantially the same apoptosis-inducing activity as the caspase.

Srinivasula et al teach constitutively active variants of caspase-3 and –6, wherein the subunits positions of the caspase are reversed and rearranged, and wherein, different from the wild types, said variants are capable of autocatalytic processing. Srinivasula et al further teach that since caspase-3 and –6 are the most downstream executioners of apoptosis, the constitutively active variants of caspase-3 and 6 could be used at very low concentration to induce apoptosis in target tissues or tumors (abstract).

US 4,753,894 teach how to make an immunotoxin comprising an antibody specific for breast cancer and a cytotoxic portion comprising ricin A chain for targeting to breast cancer cells.

Colussi PA et al, 1998, JBC, 273(41) : 26566-26570, teach that unlike an initiator caspase such as procaspase-2, an effector caspase such as procase-3 is a poor inducer of cell death, when transfected into mammalian cells, presumably because of its inability to autoactivate (p.26566, second column, second paragraph), and that artificially induced procaspase-3 oligomerization was necessary for its activation (p.26570, first column). Colussi et al further teach that fusion of procaspase-3 to the caspase-2 domain, which confers dimerization of procaspase molecules, converses procaspase-3 to an autoactivating caspase (abstract), i.e. a molecule effective in inducing apoptosis. Colussi et al also teach that apoptosis inhibitors such as MIHA and P35 inhibit the induction of apoptosis by said fusion protein of procaspase-3 to an oligomerizing domain of caspase-2 by inhibiting procaspase processing, and that Bcl-2

does not inhibit the processing of said fusion protein, because Bcl-2 acts upstream of caspase-3 by inhibiting the activation of caspase-9 (p.26569, first column).

Keppler-Hafkemeyer A et al teach that ricin A from a conjugate of ricin A-antibody could induce apoptosis of target cells via activating a caspase, besides killing cells via arresting protein synthesis caused by inactivation of the elongation factor 2.

It would have been *prima facia* obvious to a person of ordinary skill in the art at the time the invention was made to make a fusion protein comprising the constitutively active variants of caspase-3 and –6 as taught by Srinivasula et al, and an antibody that specifically binds to a tumor cell, using the method taught by US 4,753,894, and for delivering the rearranged caspase-3 or 6 taught by Srinivasula et al to target cells, because of the following reasons: 1) The constitutively active variants of caspase-3 and 6 could be used at very low concentration to induce apoptosis in target tissues, as taught by Srinivasula et al, 2) Since caspase-3 and –6 are the most downstream executioners of apoptosis, a fusion protein comprising caspase-3 would not be inhibited by an apoptosis inhibitor, such as Bcl-2, as taught by Colussi et al, and 3) The constitutively active variants of caspase-3 and 6, as taught by Srinivasula et al, are used and not the wild type caspase-3 or an initiator caspase such as caspase-9, because of the following reasons: An effector caspase such as wild type procase-3 is a poor inducer of cell death, when transfected into mammalian cells, presumably because of its inability to autoactivate, as taught by Colussi et al. Further, while caspase-3 *per se* is not the target of the apoptosis inhibitor Bcl-2, the initiator caspase, such as caspase-9 is the target of the apoptosis inhibitor Bcl-2, as taught by Colussi et al, which could

counteract the action of the initiator caspase-9. In addition, the variant of capase-3 taught Srinivasula et al would be advantageous, because it is capable of autoactivation, i.e., already has the conformation necessary for its induction of apoptosis, and does not require further oligomerization, whereas wild type caspase-3 could be inhibited by apoptosis inhibitors such as MIHA and P35, which inhibit the induction of apoptosis by inhibiting procaspase processing, i.e. oligomerization of caspase-3, as taught by Colussi et al, and 4) It would have been obvious to replace ricin A, an apoptosis inducing compound, with another apoptosis inducing compound, i.e. the constitutively active caspase taught by Srinivasula et al, because using antibody for delivering a cytotoxic compound for killing target cells is well known in the art.

One of ordinary skill in the art would have been motivated to make an antibody conjugate of the constitutively active caspase taught Srinivasula et al with a reasonable expectation of success in making such a conjugate, and in using said conjugate for killing target cells in vitro, since the constitutively active caspase taught Srinivasula et al could be used at a very low concentration to induce apoptosis in target cells, as taught by Srinivasula et al and since a conjugate of antibody-ricin A could also kill target cells by apoptosis, as taught by Keppler-Hafkemeyer A et al.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

SUSAN UNGAR, PH.D
PRIMARY EXAMINER

MINH TAM DAVIS

August 20, 2003